Effects of Compression on Intervertebral Disc Cell Gene Expression

Introduction: The intervertebral disc is the major compression carrying component of the spine, with the nucleus experiencing compressive stress and the annulus experiencing tensile stress. Excessive compression has been implicated in animal models as a causative agent for disc degeneration. In the nucleus pulposus, high compressive forces result in loss of structural proteoglycans and increased expression of matrix proteases. In addition to the destructive effects of excessive mechanical forces, it is clear that beneficial effects of motion and load also exist. For example, in animal models of dynamic loading, an anabolic response has been observed in response to moderate levels of compression. However, the magnitudes and conditions under which mechanical forces initiate trauma versus healing are not well understood. Defining and understanding these thresholds are critical to develop exercise based protocols and surgical interventions designed to optimize beneficial loading patterns. Similar to the classic studies of Nachemson, new in vivo measurements demonstrate that loading prone results in a measurement of 0.1 MPa at the nucleus pulposus, and lifting a 20kg weight with a round flexed back 2.3 MPa. As 1 MPa has been shown to have effects on matrix gene expression in annulus fibrosus, we examined loads above and below this level. We hypothesized that different magnitudes of compression would result in alterations in expression of key genes involved in disc matrix homeostasis.

Methods: We investigated gene expression in response to various magnitudes of compressive forces in an ex vivo system of healthy rabbit nucleus pulposus in order to understand how mechanical signaling regulates gene expression involved in intervertebral disc degeneration. Isolation of rabbit intervertebral disc cells: Nucleus pulposus cells were isolated from intervertebral discs from skeletally mature New Zealand White Rabbits immediately after sacrifice. Nucleus pulposus cells were cultured in 1.2 % alginate beads at a density of 4 x 10⁶ cells/ml and incubated in F-12, 10% FBS, 1% P/S at 37°C and 5% CO₂.

Application of compressive stress: Compressive stress was applied using a custom designed compression chamber capable of imparting 0-20 MPa of hydrostatic compressive load. The system utilizes compressed nitrogen pumped into a lower chamber separated from the sample chamber by a flexible diaphragm composed of Ultra-Strength Buna-N Rubber Plain Back, 1/16" Thick, 4" W, 36" L, 50A Durometer (see Figure 1). Nucleus pulposus cells in alginate beads were mounted in the system enclosed in dialysis tubing (2000 MWCO) filled with sterile media in a standard tissue culture incubator at 37°C, 5% CO₂. Static compression was applied at 0.7, 2 and 4 MPa for 4 or 24 hours. Control beads were housed in dialysis tubing and incubated in analogous culture media. All outcomes were normalized to the housekeeping gene GAPDH. Values represent the average of 5 trials +/- standard error, with 95% confidence interval calculated to determine significance.

Results: Cell viability after exposure to compressive stress up to 600 psi (4 MPa) was 80%, equivalent to that of control. Low levels of compression (0.7MPa) resulted in inhibition of expression of all genes tested (iNOS, MMP-3, TIMP-1, aggrecan). Moderate compression (2 MPa) inhibited expression of catabolic genes (iNOS and MMP-3) and enhanced anti-catabolic gene expression (TIMP-1), but also inhibited aggrecan expression. High levels of compression (4 MPa) stimulated gene expression of all genes (MMP-3, TIMP-1 and aggrecan) except iNOS (see Figure 2).

Discussion. These data demonstrate that the cells of the nucleus pulposus exhibit differential gene expression in response to different loading magnitudes. In addition, these data demonstrate beneficial effects of short term physiologic compression, and detrimental effects of prolonged loading at the same magnitude. These results suggest that while traumatic levels of loading promote catabolic activity in the disc cells, beneficial levels of loading exist which have the potential to be exploited therapeutically. Future studies will examine the net effect of these gene expression changes on matrix homeostasis as well as the relationship to in vivo levels of compression.

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